New Mycalolides from the Marine Sponge *Mycale magellanica* and Their Interconversion[1]

Shigeki Matsunaga, Takeo Sugawara, and Nobuhiro Fusetani*

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

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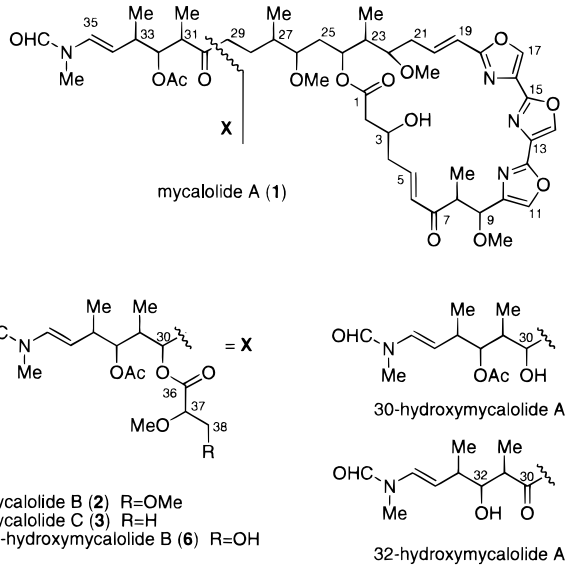
Three new macrolides, 30-hydroxymycalolide A (**4**), 32-hydroxymycalolide A (**5**), and 38-hydroxymycalolide B (**6**), were isolated from the marine sponge *Mycale magellanica*. Their structures were assigned on the basis of spectroscopic data. They were cytotoxic against L1210 cells with IC_{50} values of 0.019, 0.013, and 0.015 μg/mL, respectively. Chemical interconversion of the known mycalolides A−C (**1**−**3**) with **4**−**6** established their stereochemical relationships.

Marine sponges of the genus *Mycale* are a rich source of nitrogenous cytotoxic secondary metabolites.[2−6] Mycalolides A−C (**1**−**3**), highly cytotoxic macrolides incorporating a trisoxazole unit isolated from a Japanese *Mycale* sp., were found to have actin-depolymerizing activity.[7] In our screening for biological activities, the extract of *Mycale magellanica* Thiele (Mycalidae) exhibited potent cytotoxicity and bioassay-directed fractionation yielded three new mycalolides together with the known mycalolides A (**1**) and B (**2**). The isolation and structure elucidation of the new compounds and their chemical interconversion are described.

The EtOH extract of the sponge *M. magellanica* was partitioned between water and ether, and the ether fraction was further partitioned between *n*-hexane and MeOH/H_2O (9:1). The activity was found in the aqueous MeOH fraction, which was subjected to ODS flash chromatography followed by ODS HPLC to afford 30-hydroxymycalolide A (**4**), 32-hydroxymycalolide A (**5**), and 38-hydroxymycalolide B (**6**), which exhibited cytotoxic activity against L1210 cells with IC_{50} values of 0.019, 0.013, and 0.015 μg/mL, respectively.

30-Hydroxymycalolide A (**4**) has a molecular formula of $C_{47}H_{66}N_4O_{14}$ as determined by HRFABMS. The ^{1}H NMR spectrum immediately revealed the presence of three singlets arising from the trisoxazole moiety and the 1:2 doublet of the formamide signals, which are characteristic of the mycalolide/kabiramide class of compounds.[8] Subsequent analysis of 2D-NMR data suggested that **4** differed from mycalolide A (**1**) by the C-30 ketone, which was replaced by a secondary alcohol in **4** (Tables 1 and 2). This was supported by considerable upfield shifts of H_2-29 and H-31 as well as the appearance of an oxygenated methine proton at 3.51 ppm (H-30). Therefore, compound **4** was 30-hydroxymycalolide A. Since the stereochemistry of mycalolide A has not yet been assigned, it was not known whether **1** and **4** had the same stereochemistry at the corresponding chiral centers. To clarify this issue, both mycalolide A and 30-hydroxymycalolide A were subjected to reduction with $NaBH_4$; 30-hydroxymycalolide A furnished the allylic alcohol **7** as the major product,[9]



while mycalolide A gave two compounds, one of which coeluted with **7** in the reversed-phase HPLC and exhibited a ^{1}H NMR spectrum superimposable on that of **7**.[10] Therefore, **1** and **4** have the same stereochemistry at all the chiral centers except for C-30.

32-Hydroxymycalolide A (**5**) had a molecular formula of $C_{45}H_{62}N_4O_{13}$, which was smaller than mycalolide by C_2H_2O. Comparison of ^{1}H and ^{13}C NMR data of **5** with those of **1** revealed the absence of the acetyl signal and an upfield shift of H-32 in **5** (Tables 1 and 2), thereby indicating that **5** had a secondary alcohol at C-32 instead of the acetoxy in **1**. Acetylation of either mycalolide A or of compound **5** furnished the diacetate **8**, allowing us to confirm that the stereochemistry of all the chiral carbons of **5** is identical with that of mycalolide A.

38-Hydroxymycalolide B (**6**) had NMR spectral features similar to those of mycalolide B (**2**). However, there was a difference in the number of *O*-methyl groups; one of the five *O*-methyl signals of mycalolide B was missing in **6** (Tables 1 and 2). Therefore, **6** was likely to be the des-*O*-methyl derivative of **2**, which was supported by HRFABMS data. Comparison of NMR data for **6** and **2** readily revealed that the C-38 carbon signal in **6** experienced an upfield shift of 9 ppm, thus

* To whom correspondence should be addressed. Tel.: +81-3-3812-2111 ext 5299. Fax: +81-3-5684-0622. E-mail: anobu@hongo.ecc.u-tokyo.ac.jp.

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Table 1. ^{1}H NMR Data (CD_3OD) for **4**−**6**[a]

H no.	4	5	6
2a	2.69 dd (3.4, 14.8)	2.69 m	2.69 dd (3.3, 14.9)
2b	2.63 dd (10.1, 14.8)	2.69 m	2.62 m
3	4.41 ddt (7.6, 10.3, 3.5)	4.43 m	4.41 ddt (7.7, 10.2, 3.5)
4a	2.64 m	2.66 m	2.64 m
4b	2.48 ddt (1.5, 15.2, 7.5)	2.51 ddt (1.6, 14.7, 7.2)	2.48 m
5	7.39 dt (16.1, 6.8)	7.39 dt (16.2, 6.6)	7.38 dt (16.1, 6.8)
6	6.17 dt (16.2, 1.6)	6.17 dt (16.2, 1.6)	6.17 dt (16.2, 1.6)
8	4.23 dq (9.4, 6.7)	4.21 dq (9.4, 6.9)	4.22 dq (9.3, 6.9)
8-Me	0.87 d (6.7)	0.88 d (6.9)	0.87 d (6.9)
9	4.35 d (9.4)	4.36 d (9.4)	4.35 d (9.3)
9-OMe	3.14 s	3.14 s	3.14 s
11	8.05 s	8.06 s	8.03 s
14	8.59 s	8.60 s	8.57 s
17	8.51 s	8.52 s	8.49 s
19	6.47 d (15.8)	6.48 dt (15.8, 1.5)	6.46 dt (15.7, 1.6)
20	7.14 ddd (15.3, 8.8, 6.1)	7.21 ddd (15.8, 9.0, 5.9)	7.13 m
21a	2.76 m	2.74 dddd (15.0, 6.3, 4.3, 2.0)	2.75 m
21b	2.51 br dt (15.8, 8.4)	2.60 m	2.50 m
22	3.46 m	3.43 dt (6.4, 4.3)	3.47 m
22-OMe	3.33 s	3.34 s	3.35 s
23	1.94 ddq (4.0, 6.8, 7.0)	2.01 ddq (4.2, 6.6, 7.0)	1.93 m
23-Me	0.93 d (7.0)	0.95 d (7.0)	0.92 d (7.0)
24	5.28 ddd (9.7, 6.8, 1.9)	5.27 m	5.27 ddd (9.8, 6.8, 1.9)
25a	1.65 m	1.63 m	1.60 m
25b	1.57 m	1.59 m	1.54 m
26	3.12 m	3.14 m	3.11 m
26-OMe	3.35 s	3.37 s	3.32 s
27	1.77 m	1.78 m	1.80 m
27-Me	0.86 d (6.5)	0.85 d (6.9)	0.85 d (6.9)
28a	1.62 m	1.77 m	1.63 m
28b	0.93 m	1.22 m	0.96 m
29a	1.45 m	2.61 m	1.61 m
29b	1.42 m	2.59 m	1.61 m
30	3.51 dt (2.0, 7.6)		5.08 m
31	1.65 m	2.65 m	1.91 m
31-Me	0.86 d (6.4)	0.97 d (6.9)	0.99 d (6.9)
32	4.93 dd (9.7, 2.9)	3.63 m	4.77 dd (10.2, 2.8)
32-OAc	2.10 s [2.09 s]		2.05 s
33	2.61 m	2.41 ddq (9.5, 2.6, 6.9)	2.64 m
33-Me	1.03 d (6.9)	1.12 d (6.9)	1.00 d (6.9)
34	5.12 dd (14.1, 9.4) [5.19 dd (14.6, 9.3)]	5.19 dd (14.1, 9.5) [5.26 dd (14.6, 9.5)]	5.08 dd (14.1, 9.5) [5.15 dd (14.6, 9.4)]
35	6.75 d (14.1) [7.12 d (14.6)]	6.70 d (14.1) [7.09 d (14.6)]	6.75 d (14.1) [7.15 m]
35-NMe	2.99 s [3.08 s]	3.01 s [3.11 s]	2.99 s [3.09 s]
35-NCHO	8.32 s [8.07 s]	8.31 s [8.08 s]	8.32 s [8.08 s]
37			3.81 dd (6.7, 3.4)
37-OMe			3.44 s
38a			3.80 dd (11.7, 3.4)
38b			3.70 dd (11.7, 6.7)

[a] Signals for the minor isomer are shown in square brackets.

suggesting that the C-38 methoxyl group in **2** was replaced by a hydroxyl group in **6**. To correlate the stereochemistry of mycalolide B with that of compound **6**, each was reduced with $NaBH_4$ followed by hydrolysis with LiOH.[11] Although the expected pentol **9** was not obtained, compound **10** was obtained in each case,[12] which indicated that both **6** and **2** had identical stereochemistry except for the unidentified chiral center C-37 in the glycerate residue. Furthermore, mycalolide C (**3**) and 30-hydroxymycalolide A (**4**) were treated in the same way to yield **10**, thereby disclosing that compounds **1**−**6** had the same stereochemistry at the corresponding chiral centers.[13]

Experimental Section

General Methods.[14] FAB mass spectra were obtained in the positive ion mode with a matrix of 3-nitrobenzyl alcohol.

Biological Material. The sponge was collected at a depth of 15 m off Kumomi on the Izu Peninsula and identified by Professor P. Bergquist as *M. magellanica* Thiele. A voucher specimen was deposited at the Laboratory of Aquatic Natural Products, The University of Tokyo (no. S1123).

Extraction and Isolation. The frozen sample (1 kg) was extracted with EtOH and the combined extract was evaporated to yield an aqueous suspension, which was partitioned between water and ether. The ether phase was partitioned between *n*-hexane and MeOH/H_2O (9:1), and the latter phase, which was antifungal against *Mortierella ramanniana* was applied to a column of ODS and eluted with aqueous MeOH by increasing the concentration of MeOH. The fractions eluted with MeOH/H_2O (8:2) and MeOH/H_2O (9:1) were combined and further chromatographed on a column of Sephadex LH-20 (MeOH). The antifungal fractions were further purified by ODS HPLC with MeOH/H_2O (7:3) followed by ODS HPLC with MeCN/H_2O (1:1) to yield 30-hydroxymycalolide A (**4**, 7.5 mg), 32-hydroxymycalolide A (**5**, 12.3 mg), and 38-hydroxymycalolide B (**6**, 14.6 mg) together with mycalolide A (**1**, 8.5 mg) and mycalolide B (**2**, 32.3 mg).

Table 2. ^{13}C NMR Data (CD_3OD) for **4**−**6**[a]

C no.	4	5	6	C no.	4	5	6
1	173.5	173.8	173.4	26-OMe	58.2	58.3	58.0
2	43.2	43.0	43.2	27	36.1	35.5	35.4
3	68.6	68.5	68.6	27-Me	16.1	15.8	16.1
4	41.5	41.2	41.6	28	29.0	25.8	28.3
5	148.2	148.0	148.3	29	34.1	42.0	31.3
6	134.6	134.6	134.6	30	71.3	217.3	74.6
7	205.6	205.6	205.6		[71.5]		
8	44.3	44.4	44.3	31	40.7	51.5	38.8
8-Me	14.4	14.5	14.4	31-Me	9.1	13.8	9.8
9	78.4	78.4	78.4	32	79.8	78.1	78.3
9-OMe	56.8	56.8	56.8	[32]		[78.0]	
10	140.0	140.2	140.0	32-OAc	173.1		172.3
11	139.9	139.9	140.0	[32-OAc]	20.9		21.0
12	157.3	157.3	157.3	33	37.9	38.5	38.0
13	132.0	131.9	132.0	[33]	[38.1]	[38.8]	[38.2]
14	139.9	140.0	139.9		19.8	19.7	19.7
15	158.2	158.2	158.1	[33-Me]	[19.6]		
16	131.1	131.1	131.2	34	112.4	112.6	111.7
17	139.9	140.0	139.9	[34]	[114.5]	[114.8]	[113.9]
18	164.3	164.3	164.3	35	131.0	130.6	131.3
19	117.6	117.8	117.6	[35]	[126.2]	[125.8]	[126.5]
20	141.3	141.2	141.3	35-NMe	27.6	27.6	27.6
21	34.6	34.4	34.7	[35-NMe]	[33.5]	[33.5]	[33.5]
22	81.3	81.7	81.3	35-NCHO	164.7	164.7	164.7
22-OMe	58.0	57.9	58.1	[35-NCHO]	[163.4]	[163.4]	[163.4]
23	41.3	41.0	41.3	36			172.7
23-Me	9.1	9.4	9.1	37			83.8
24	74.6	74.8	74.6	37-OMe			59.0
25	33.1	32.8	32.9	38			64.0
26	83.1	83.0	82.8				

[a] Signals for the minor isomer are shown in square brackets.

30-Hydroxymycalolide A (4): $[\alpha]^{27}_D$ −86.9° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 230 nm (ϵ 27 000); ^1H NMR data in CD_3OD, see Table 1; ^{13}C NMR data in CD_3OD, see Table 2; HRFABMS *m/z* 933.4473 (calcd for $C_{47}H_{66}N_4O_{14}$-Na, 933.4473).

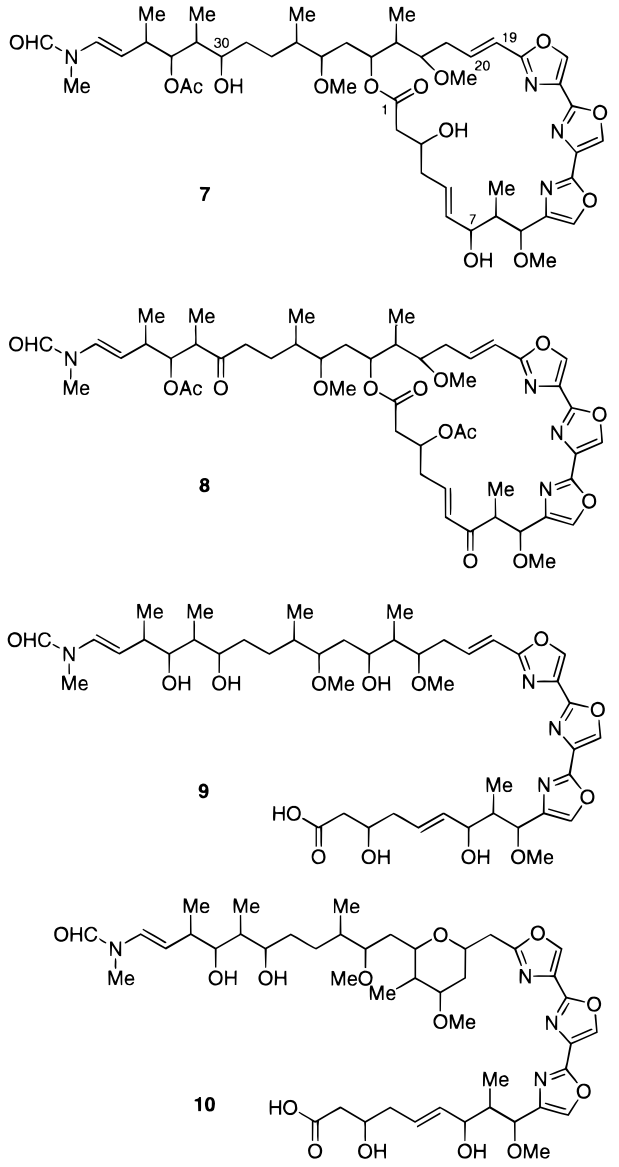
32-Hydroxymycalolide A (5): $[\alpha]^{27}_D$ −90.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 230 nm (ϵ 26 000); ^1H NMR data in CD_3OD, see Table 1; ^{13}C NMR data in CD_3OD, see Table 2; HRFABMS *m/z* 889.4212 (calcd for $C_{45}H_{62}N_4O_{13}$-Na, 889.4214).

38-Hydroxymycalolide B (6): $[\alpha]^{27}_D$ −80.9° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 230 nm (ϵ 25 000); ^1H NMR data in CD_3OD, see Table 1; ^{13}C NMR data in CD_3OD, see Table 2; HRFABMS *m/z* 1035.4805 (calcd for $C_{51}H_{72}N_4O_{17}Na$, 1035.4820).

$NaBH_4$ Reduction of 30-Hydroxymycalolide A (4) and Mycalolide A (1). To a solution of 30-hydroxymycalolide A (1.7 mg) in MeOH (0.5 mL) was added $NaBH_4$ (2.2 mg) and the mixture stirred for 30 min at 0 °C. To the reaction mixture was added 5% AcOH in H_2O (1 mL), and the solution was subjected to ODS−HPLC with MeOH/H_2O (8:2) to yield **7** (1.6 mg). Mycalolide A (1.7 mg) was similarly treated to afford **7** (0.9 mg) and the C-30 epimer of **7** (0.6 mg).

Compound 7: FABMS (3-nitrobenzyl alcohol supplemented with NaCl) *m/z* 935 $(M + Na)^+$. For the ^1H NMR spectrum, see the Supporting Information.

Acetylation of 32-Hydroxymycalolide A (5) and Mycalolide A (1). Compound **5** (1 mg) was dissolved in a 1:1 mixture of acetic anhydride and pyridine (1 mL) and the mixture stirred overnight at room temperature. After removal of the solvent by lyophilization, the reaction product was applied to a silica gel column (1 × 2 cm) and eluted with $CHCl_3$ followed by a mixture of $CHCl_3$/MeOH (98:2) to furnish the diacetate **8** in quantitative yield. Mycalolide A was treated in the same way to afford **8**.



Compound 8: FABMS (3-nitrobenzyl alcohol supplemented with NaCl) *m/z* 973 $(M + Na)^+$. For the ^1H NMR spectrum, see the Supporting Information.

Base Hydrolysis of Mycalolide B (2), Mycalolide C (3), 30-Hydroxymycalolide A (4), and 38-Hydroxymycalolide B (6). Mycalolide B (0.8 mg) was reduced with $NaBH_4$ as described above, and the major peak in the reversed-phase HPLC was collected. The reduction product was dissolved in a mixture of MeOH/1 N LiOH in H_2O (2:1, 1 mL), and the mixture was allowed to stand at room temperature overnight. To the reaction mixture was added AcOH (100 μL), and after removal of MeOH by evaporation, the aqueous solution was subjected to ODS HPLC with MeOH/H_2O (7:3) to furnish **10** (0.6 mg). Mycalolide C, 30-hydroxymycalolide A, and 38-hydroxymycalolide B were treated in the same way to yield **10**, whose ^1H NMR spectrum was indistinguishable from the spectrum of the compound prepared from **2**.

Compound 10: FABMS (3-nitrobenzyl alcohol supplemented with NaCl) *m/z* 911 $(M + Na)^+$, 933 $(M + 2Na - H)^+$. For the ^1H NMR and COSY spectra, see the Supporting Information.

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Supporting Information Available: Copies of ^{1}H NMR spectra of compounds **7**, **8**, and **10** and the COSY spectrum of **10** (4 pages). Ordering information is given on any current masthead page.

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(9) Minor products are (1) the C-1 alcohol of **7**, (2) the C-7-epimer of **7**, and (3) the C19,C20-saturated derivative of **7**, which were characterized by FABMS and ^{1}H NMR data (data not shown).
(10) Although not subjected to NMR analysis, the other product [*m*/*z* 935 $(M + Na)^+$ in the FABMS] is most likely the C-30-epimer of **7**.
(11) It was necessary to reduce the C-7 ketone to accomplish a clean conversion.
(12) Production of compound **10** is rationalized as intramolecular Michael-type addition of the C-24 alcohol to the C-19−C-20 unsaturated olefin. In compound **10**, the olefinic signals for H-19 and H-20 were replaced by low-field methylenes at δ 3.04 (H-19a), 3.22 (H-19b), and an oxygenated methine at 4.25 (H-20).
(13) Without chiroptical data interconversion among **1**−**6** only reveals the identity of relative stereochemistry.
(14) For experimental details, see ref 4.

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